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U. S. DEPARTMENT OF AGRICULTURE.

Report No. 68.

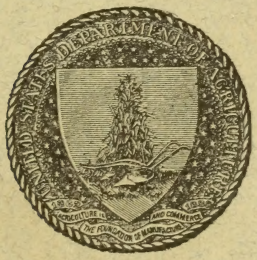
CATALASE,

A NEW ENZYM OF GENERAL OCCURRENCE,

WITH SPECIAL REFERENCE TO THE TOBACCO PLANT.

BY

OSCAR LOEW,
Division of Vegetable Physiology and Pathology,
Detailed to the Division of Soils.



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Bulletin No. 11, Division of Soils.—Tobacco Soils of the United States, a Preliminary Report upon the Soils of the Principal Tobacco Districts. By Milton Whitney, Chief of Division of Soils.

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Report No. 65.—Physiological Studies of Connecticut Leaf Tobacco. By Oscar Loew, of the Division of Vegetable Physiology and Pathology.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Division of Soils,
Washington, D. C., September 28, 1900.

Sir: I have the honor to transmit herewith a manuscript on a new enzym occurring in the tobacco plant, and probably having much to do with the fermentation and, possibly, with the development of flavor and aroma in tobacco. It represents work done in cooperation with the Division of Vegetable Physiology and Pathology of this Department.

Respectfully,

Milton Whitney,
Chief of Division.

Hon. James Wilson,
Secretary of Agriculture.

LETTER OF SUBMITTAL.

U. S. Department of Agriculture,
Division of Vegetable Physiology and Pathology,
Washington, D. C., September 28, 1900.

Dear Sir: In continuation of the cooperative work of this Division and the Division of Soils, I hand you herewith a paper prepared by Dr. Oscar Loew, on catalase, a new enzym of general occurrence, with special reference to the tobacco plant. The paper is the result of investigations carried on in connection with the tobacco work in your charge.

I regret very much to state that this will be the last of the series of important papers by Dr. Loew which will be brought out in cooperation with your Division, as he has just accepted a position in the Imperial University at Tokyo, Japan.

Respectfully,

B. T. Galloway,
Chief of Division.

Prof. Milton Whitney,
Chief Division of Soils,
In charge of Tobacco Investigations.

TWO KINDS OF CATALASE.

It was one of the first observations of the writer when he commenced the study of the new enzym that it existed in an insoluble and in a soluble form. These may be distinguished as α- and β-catalase, respectively. The former is probably a compound of the soluble catalase with a nucleo-proteid, while the soluble catalase is an albumose and can be liberated by the action of very dilute alkaline media upon the insoluble catalase. This conversion can be observed well in the process of sweating tobacco in bulk, whereby the temperature gradually reaches 60° C., and ammonium carbonate is generated as one of the products of the destructive processes going on. Cured tobacco contains but little soluble catalase, while well-sweated tobacco contains much. For example, it was observed that the cold-prepared aqueous extract of 2 grams of cured tobacco yielded in thirty minutes, on the addition of dilute hydrogen peroxid in excess, only 16.5 cc. of oxygen, while the same tobacco after sweating in bulk yielded 236 cc. under similar conditions.

Sometimes sweated tobacco proves rich in β-catalase, as may be inferred from the following test. Five grams of finely powdered tobacco (Zimmer Spanish) were digested for twenty-four hours with 500 cc. of cold water, with the addition of some chloroform. Twenty cc. of this highly diluted extract yielded in thirty minutes, upon the addition of 5 cc. hydrogen peroxid, 34 cc. oxygen.[1]

Crude β-catalase is obtained by preparing at the ordinary temperature a concentrated extract from tobacco sweated in bulk, with water containing chloroform, using the same water for extracting three or four portions of the tobacco. This extract is saturated at the ordinary temperature with ammonium sulphate. The precipitate thus obtained is dried at the room temperature between sheets of filter paper, which removes most of the adhering salt solution. This crude product contains, among other substances, besides adhering ammonium sulphate, a brown coloring matter which can not be removed by digesting the solution with boneblack, since this absorbs the dissolved enzym also. The ammonium sulphate can be removed by osmosis, and the enzym may then be precipitated by alcohol. The activity is better preserved, however, when the fresh moist precipitate is dissolved in some glycerol. Colorless products can be prepared from the seeds of clover or poppy; but these preparations are weaker than that from sweated tobacco, since they contain more impurities, among which may be found a little peroxidase. A very energetic product may be

[1] The hydrogen peroxid preparation mentioned in this bulletin refers to the commercial article. It contained from 2.8 to 3.1 per cent pure hydrogen peroxid. The weak acid reaction was removed immediately before use by diluted sodium hydrate or carbonate. A preparation yielding ten times its volume of oxygen by catalysis contains 3.04 per cent of pure peroxid by weight.

obtained from culture fluids of *Penicillium glaucum* in the manner above described. The crude β-catalase from animal organs contains more proteins as impurities than that from sweated tobacco.

While β-catalase[1] behaves like an albumose, α-catalase behaves like a nucleo-proteid, since it is not soluble in water alone. It can be brought into solution by dilute alkaline liquids and precipitated again therefrom unchanged on the addition of acetic acid.[2] Sweated tobacco was finely pulverized and repeatedly extracted with a large amount of cold water, to which a few drops of ether had been added in order to prevent bacterial growth, and then treated for fifteen hours with a 0.2 per cent solution of caustic soda. This alkaline extract, after filtering, was cautiously acidulated with acetic acid (mere neutralization does not yield a precipitate), and the precipitate filtered off after standing one hour. This precipitate possessed, in a considerable degree, the power of decomposing hydrogen peroxid, while the liquid filtered from it did not show this power in the least. This behavior was also observed with green tobacco leaves. Six hundred square centimeters of healthy tobacco leaves from the greenhouse were crushed to a fine pulp with sand and left for three hours in contact with a 0.2 per cent solution of ammonium carbonate. The opalescent filtrate obtained had in a high degree the power of catalyzing hydrogen peroxid. Upon adding dilute acetic acid until a slight acid reaction was reached, a fine flocculent precipitate containing the active principle was formed, the filtrate from this precipitate being devoid of the catalyzing property.

The α-catalase is, however, not very readily extracted by alkaline liquids. Even a 1 per cent solution of sodium carbonate dissolves it but slowly. The solution thus obtained from sweated tobacco that had been well exhausted with water before the treatment with sodium carbonate was begun does not give the characteristic blue reactions for oxidase and peroxidase (a previous slight acidulating of the alkaline liquid is necessary for these tests), hence α-catalase also has no close relation to these two oxidases. In these tests 10 per cent alcohol was added to the extracting liquids to prevent bacterial growth during the time of extraction.

Connecticut tobacco, cured in 1899, was repeatedly extracted with water saturated with chloroform. After the removal of the soluble compounds this tobacco was left for two days with a 0.5 per cent solution of sodium carbonate, just enough to cover the soaked tobacco with the solution. Then the liquid was separated from the tobacco and 20 cc. of the brown-colored filtrate mixed with 5 cc. hydrogen peroxide, and in fifteen minutes 7.5 cc. of oxygen were obtained. A

[1] Special tests have shown that it is not a coagulable albumin.

[2] This militates against the supposition that α-catalase might be merely β-catalase in a state of absorption, the same way that colored matters are absorbed by charcoal.

quantity of the expressed residue corresponding to nearly 2 grams of dry matter, after being washed well and again expressed, was suspended in 20 cc. water and 10 cc. hydrogen peroxid added; in fifteen minutes 31 cc. of oxygen were obtained. This shows that a solution of 0.5 per cent sodium carbonate did not extract all the α-catalase even in two days.

It is more difficult to prepare the α-catalase free from peroxidase when using green tobacco leaves than when cured and fermented leaves are used, since it takes a long time to wash out the peroxidase from the pulp prepared from the fresh leaves. For example, 30 cc. of turbid, not filtered, juice prepared from tobacco leaves was mixed with ten times this quantity of water and filtered after standing fifteen hours, leaving a green residue in the filter, which even after prolonged washing yielded the reaction for peroxidase, while that for oxidase had ceased to be produced in the wash waters after a short time.

Water alone dissolves only traces of α-catalase at 20° C., while prolonged action of water at a moderate elevation of temperature gradually splits off β-catalase, the process being favored by adding a small proportion of sodium carbonate.

The following test shows that even very large quantities of water alone do not extract α-catalase to any noticeable extent. Two grams of finely pulverized cured tobacco were extracted for four hours with 40 cc. of water at 20° C. and the residue washed until the filtrate was nearly colorless. This extracted tobacco yielded in fifteen minutes, upon the addition of 5 cc. hydrogen peroxid and 20 cc. water, 42 cc. oxygen. Two grams of the same tobacco were now extracted with 1 liter of chloroform water for eighteen hours. The powder yielded, after washing under the same conditions as before, 40 cc. oxygen.

It seems strange that neither Schœnbein nor Jacobson observed that the property of catalyzing hydrogen peroxid may be due to a soluble as well as to an insoluble substance. Schœnbein mentions only once—without, however, altering his opinion that the enzyms were the cause of that catalysis—that the filtered extracts of certain vegetable matter show much less power of catalyzing hydrogen peroxid than the unfiltered. Jacobson also used, in his experiments mentioned above, the crude turbid extracts, but not purified enzym preparations. He used, for instance, an emulsion prepared from crushed almonds instead of purified emulsin.

The following tests leave no doubt that the transformation from the insoluble to the soluble catalase really can take place. Sweated tobacco rich in α-catalase was finely pulverized, repeatedly extracted with chloroform water[1] at the ordinary temperature, and, when β-catalase

[1] Care must be taken in the application of chloroform water as an antiseptic that a full saturation is preserved, otherwise certain microbes will gradually develop.

could no longer be extracted, heated with a 1 per cent solution of sodium carbonate for one hour at 40° C. The mixture was passed through flannel and the filtrate moderately acidulated with acetic acid to precipitate nucleo-proteids. The clear filtrate now obtained had a considerable power of catalyzing hydrogen peroxid, although it could not contain α-catalase any longer.

In a second trial the exhausted tobacco was heated for two hours at from 45° to 50° C., with a solution of 0.2 per cent sodium carbonate, the filtrate acidulated with acetic acid, again filtered, and the filtrate now saturated with ammonium sulphate at the ordinary temperature. By this treatment the active principle was salted out, as is the case with β-catalase, the precipitate showing powerful action while the filtrate showed only slight traces of activity. It may also be stated that the salted-out product was easily soluble again in water and can not therefore have consisted of nucleo-proteids.

Since there is a zymogen for every well-known enzym, the supposition seems justifiable that there is also a zymogen of catalase. Various observers have shown that animal or vegetable cells, before they form the enzyms, are rich in small granules, which disappear in a measure as the enzyms are formed. The substance first secreted by the nucleus, forming the granules in the cytoplasm, is distinguished by Macallum as pro-zymogen. Vines, in his investigation on the presence of diastase in leaves, observed that the turbid unfiltered extracts saccharified more starch than the clear filtered ones. Brown and Morris found, furthermore, that powdered leaves are more powerful in diastasic action than an aqueous extract prepared from the same amount of leaf. Whether the action here is caused by a zymogen which is gradually transformed into diastase, or whether the diastase itself is retained to a certain extent by the solid compounds of the leaf, has not been decided. It has been observed that highly dilute acids bring on the transformation of zymogen into enzym. J. Reynolds Green has found that certain rays of light—located chiefly in the red, orange, and blue regions of the spectrum—and a prolonged exposure to a temperature of 38° C. can also cause this change.[1]

The existence of a zymogen of catalase in tobacco leaves appears probable from an observation of the writer that in the process of sweating tobacco in bulk at a temperature of from 40° to 50° C. the power of catalyzing hydrogen peroxid is increased, not only for the soluble but also for the insoluble catalase. Digestion with 0.1 per cent oxalic acid at from 25° to 36° C. proved a failure in this direction, since the catalytic power was decreased thereby. Acids therefore seem unsuited to transform zymogen into enzym in the case of catalase.

[1] Philosoph. Transactions, vol. 188 (1897). On the other hand, diastase itself is gradually destroyed by white light, as the same author has observed.

EFFECTIVENESS OF THE β-CATALASE.

For the following tests a preparation of crude β-catalase was used which was obtained by salting out with ammonium sulphate a concentrated cold-prepared extract of tobacco recently sweated in bulk. The precipitate, after being pressed between filter paper, was permitted to dry at the room temperature. A 1 per cent solution of this crude β-catalase, still containing a considerable amount of ammonium sulphate, was prepared. One cc. of this solution was mixed with 20 cc. of water and 2 cc. of common hydrogen peroxid (neutralized). The volume of oxygen evolved for different lengths of time was as follows:

	cc.
After 5 minutes	12
After 10 minutes	17
After 16 minutes	20.2

This result shows that in the dilution of 1:2,000 the crude enzym decomposed six times its own weight of anhydrous hydrogen peroxid in sixteen minutes.

In the next test the reaction of the mixture was kept slightly alkaline and the amount of hydrogen peroxid increased to 10 cc., the other conditions being the same as before:

	cc.
After 5 minutes	20
After 10 minutes	38
After 15 minutes	55
After 40 minutes	76
After 55 minutes	80
After 15 hours	101.5

In a further test 1 cc. of the 1 per cent catalase solution mentioned above was diluted with 200 cc. water and 1 cc. hydrogen peroxid added. One minute after the mixture was made the reaction for hydrogen peroxid was still very powerful.[1] After twenty-five minutes, however, the reaction was very weak, and after thirty-two minutes there was no trace of hydrogen peroxid left. This test was repeated with the modification that the amount of water was increased to 500 cc. and the amount of hydrogen peroxid decreased to 0.5 cc. After twenty-one minutes the reaction for hydrogen peroxid was very much weaker than in the beginning, and after fifty minutes no reaction at all was obtained. This shows that even in a dilution of 1:50,000 the crude enzym was still very active. In the control case, where the solution of the enzym was boiled for a second, the reaction for hydrogen peroxid did not decrease to any noticeable degree within the same length of time.

While catalase attacks hydrogen peroxid very readily, the hydrogen peroxid also has, when applied in moderate concentration, a directly

[1] The test was made with iodide of potassium, starch, and a trace of ferrous sulphate.

oxidizing influence upon the enzym. This accounts for the rapid decrease of the amount of oxygen developed and for the fact that a point is reached comparatively soon where the enzym becomes entirely inactive. Sweated tobacco, 0.1 gram of which developed 32 cc. of oxygen in fifteen minutes when suspended in a mixture of 10 cc. water with 5 cc. hydrogen peroxid, did not develop any more after the same quantity had been left for fifteen hours in contact with 25 cc. of the same mixture. It may safely be assumed that the detrimental effect of hydrogen peroxid is diminished very much by high dilution.

The activity of β-catalase increases up to a temperature of about 40° C. With further elevation of the temperature the oxidizing action of hydrogen peroxid upon the enzym becomes more powerful than the catalytic action of the enzym upon the hydrogen peroxid, and the enzym very soon loses its activity. A solution was prepared of 0.2 per cent crude β-catalase obtained from sweated tobacco in the manner above described. Thirty cc. of this solution were heated to determined temperatures, and after addition of 5 cc. of hydrogen peroxid of 20° C. the time required for the development of a certain volume of oxygen was noted. At 50° and 60° C. the action was energetic for one minute only, after which it slowed down rapidly and stopped.

Effect of heat on action of β-catalase.

Temperature.	Duration of test.	Volume of oxygen.	Remarks.
° C.	*Minutes.*	*cc.*	
20.....	5½	40	
40.....	3½	40	
50.....	4	29	Action stopped after 4 minutes.
60.....	2	23	Action stopped after 2 minutes.

GENERAL BEHAVIOR OF α- AND β-CATALASE.

Both the soluble and the insoluble catalase resist the effects of time longer than the ordinary oxidases, as has already been mentioned. The writer examined leaves of various species of *Solanum*, collected in 1841, 1848, 1853, and 1868 (preserved as herbarium specimens), and observed in all of these the production of oxygen upon the addition of hydrogen peroxid. The leaves were first soaked in a little water before the hydrogen peroxid was added. The leaves collected in 1841, however, showed only a weak action.

In an aqueous solution, free from bacteria, β-catalase loses its power either by self-oxidation or by changes within the molecule by atomic migration, but this change is also sometimes relatively slow. Once, however, the writer observed this enzym in a six-months-old culture of *Bacillus pyocyaneus*, in which growth of the germ had apparently stopped for several months.

In aqueous solution the β-catalase is chemically changed to an inac-

tive substance on heating to from 71° to 75° C. The degree of temperature at which the enzym is "killed" depends upon the time of exposure. At 72° C. more than fifteen minutes are required, while a much shorter time suffices at 74° C. Heating but for a second to 75° is not sufficient to kill all the enzym in a neutral solution, but it will injure a great part of it. In a very weak alkaline solution the killing temperature is raised several degrees, but the contrary is true when this alkaline reaction is increased still a little more. In slightly acidulated solutions the killing temperature is lower than in neutral ones.

The killing temperature of α-catalase is about the same as that of β-catalase. Green tobacco leaves were crushed and the juice expressed through flannel. This turbid liquid of acid reaction was passed through a filter, and the filter content, rich in α-catalase, suspended in water and heated to various temperatures. Heating for ten minutes to 60° C. did not decrease the intensity of the catalytic power, but heating for five minutes to 71° C. injured it considerably. After heating for five minutes to 75° C. and cooling quickly a faint trace of that power was still observed, but no trace was noticeable after heating for one minute to 80°. The filtered liquid in the latter case still contained considerable active peroxidase, another proof that this enzym can not be identified with catalase.

In a dry condition catalase resists heat better than in the presence of water. For example, fermented tobacco leaves when heated for two hours to 100° C. had not completely lost their content of α- and β-catalase, although it was very much decreased. A prolonged application of heat, however, kills these enzyms at 80° C., even in the absence of water, as may be inferred from the inactivity of fire- and flue-cured tobacco (cigarette tobacco). Various samples of such products were tested, and in most cases no trace of either α- or β-catalase could be observed.

EFFECT OF MECHANICAL MOTION ON THE REACTION WITH HYDROGEN PEROXID.

It is remarkable to what extent the action of the soluble catalase is promoted by shaking the mixture. One out of many of the observations may be mentioned here. A cold-prepared aqueous extract of sweated tobacco (20 cc.) was mixed with half its volume of hydrogen peroxid and the flask left untouched. In the control case the flask was continuously shaken. The amount of oxygen developed in thirty minutes, not shaken, was 24 cc.; shaken, was 51 cc. The oxygen developed under the influence of mechanical motion was therefore more than double the amount obtained when the flask was left undisturbed.[1]

[1] It hardly needs to be mentioned that in all experiments described in this paper the mixtures with hydrogen peroxid were frequently shaken during the observations in order to obtain the full reaction. Heating the mixtures was never resorted to; on the contrary, any rise of temperature was avoided.

BEHAVIOR TOWARD VARIOUS SALTS.

A careful distinction should be made here between the relation of a salt to the enzym itself and the influence of a salt upon its activity alone. This activity can be stimulated or retarded without the enzym itself being changed in its chemical nature. Salts of a strong acid or alkaline reaction will of course injure the enzym itself, while salts of neutral reaction will probably not do so. Thus, α-catalase is not injured in one day by a 10 per cent solution of magnesium sulphate, nor in five hours by a 5 per cent solution of sodium sulphate, nor in twenty hours by a 2 per cent solution of potassium persulphate,[1] nor in one day by a saturated solution of ammonium sulphate. After these salts are removed by careful washing the α-catalase contained in sweated tobacco is found fully preserved, or very nearly so. It is very probable that β-catalase is more easily injured, but nevertheless it can even be salted out by saturation of its solution with ammonium sulphate without immediate loss of power, although the subsequent drying at the ordinary temperature with the adhering salt leads to a partial loss of its activity.

A concentrated extract was prepared at the ordinary temperature from sweated tobacco (Connecticut crop of 1899) rich in α- and β-catalase, and containing no oxidase and only a trace of peroxidase. Ten cc. of this extract were mixed with 20 cc. of hydrogen peroxid (added in two doses), and in five minutes 160 cc. of oxygen were developed. By saturation with ammonium sulphate a brown-colored precipitate of great catalytic energy was obtained, while the filtrate showed only a trace of activity. After being collected on a filter and dried for twenty-four hours at the ordinary temperature on a porous plate, the precipitate weighed 2.85 grams, more than half of which, however, consisted of adhering ammonium sulphate. Half a gram of this powder dissolved in 20 cc. of water in five minutes developed 223 cc. oxygen, and in fifteen minutes 449 cc.[2]

The considerable depression of the catalytic activity of β-catalase by nitrates is very remarkable. The enzym itself does not seem to be injured materially, as several tests have shown. For example, a very small quantity of a β-catalase preparation, obtained by salting out a cold-prepared concentrated tobacco extract with ammonium sulphate, was kept in a 10 per cent solution of potassium nitrate for twenty-

[1] It might be supposed that this salt would exert an oxidizing action detrimental to the enzym, but such indifferent behavior of the salt has been noticed in other cases also; for example, toward certain aniline colors. (Proscher, Chem.-Zeitung, March, 1900.) Potassium persulphate easily oxidizes hydroquinone to quinone, α-naphthylamine to the blue oxynaphthylamine, and behaves in its oxidizing property like ferric chlorid.

[2] The observation of Jacobson that the "catalytic power" of a pancreas extract, or of an emulsion of almonds, is lost by saturation with sodium sulphate, is probably due to his drying the salted-out product at a higher temperature.

four hours, again salted out by ammonium sulphate, and washed well by a concentrated solution of this salt. Upon redissolving it in 20 cc. water and adding 10 cc. hydrogen peroxid, it still developed 92 cc. oxygen in fifteen minutes, while in the control case 98 cc. were obtained; hence the enzym itself is not injured by potassium nitrate.

The depressing influence of nitrates is seen from the following test: Two portions of an extract of sweated tobacco received an addition of 5 per cent potassium nitrate and 5 per cent magnesium sulphate (cryst.), respectively. Immediately afterwards 20 cc. of each of these liquids were mixed with 10 cc. hydrogen peroxid. The result was:

Oxygen developed in five minutes.

	cc.
In presence of potassium nitrate[1]	15
In presence of magnesium sulphate	88
Control	105

Nitrates have no such depressing influence upon the corresponding action of finely divided platinum. A mixture was prepared of 5 cc. hydrogen peroxid, 20 cc. water, 2 grams potassium nitrate, and a trace of platinum black. Here 28 cc. oxygen were developed in fifteen minutes, while in the control case 31.6 cc. were evolved. This shows that the contact of the nitrate with the enzym itself can be the only cause of the retardation just mentioned.

In some other tests the time necessary to develop a certain volume of oxygen was observed. One gram of crude β-catalase, prepared by salting out a concentrated extract of sweated tobacco and drying at the ordinary temperature, was dissolved in 500 cc. water. To different portions, each containing 5 cc., of this highly diluted solution, 20 cc. water and 0.5 gram of various salts were added. After a further addition of 5 cc. of nearly neutral hydrogen peroxid the time necessary for the production of 40 cc. of oxygen was observed. The mixture was frequently shaken. The number of minutes required to produce 40 cc. of oxygen in the presence of various salts was as follows:

	Minutes.
With sodium chlorid	$4\frac{3}{4}$
With potassium chlorid	$4\frac{3}{4}$
With dipotassium phosphate	$4\frac{3}{4}$
With potassium nitrate	$8\frac{1}{2}$
With sodium nitrate	$6\frac{1}{2}$
With ammonium nitrate	$10\frac{1}{2}$
With calcium nitrate	$3\frac{3}{4}$
Without addition of salts—control	$4\frac{1}{2}$

While in this case the mixtures had a weak acid reaction, they had in the following experiment a weak alkaline reaction. The enzym, furthermore, was applied in double the concentration used before, and, with one exception, the amount of salts applied was increased to

[1] The depression of the catalytic power of pancreas and almond extract by potassium nitrate was also observed by Jacobson in 1892.

2 grams. Here the time required to produce 48 cc. of oxygen under otherwise the same conditions as before was noted.

The number of minutes required to produce 48 cc. of oxygen in the presence of various salts was as follows:

	Minutes.
With sodium chlorid	3
With potassium chlorid	3¾
With potassium nitrate	4½
With sodium sulphate	3
With potassium sulphate	4
With sodium carbonate (0.5 gram)	2
With sodium carbonate (2 grams)	4¼
Without addition of salts—control	3

It will be noticed that potassium salts retard the reaction more than sodium salts, and that the nitrates of the alkali group retard more than the other salts. In general, the increase of the amount of any salt has a retarding influence, other things being equal, and it was found in a special case that saturation of the liquid with sodium chlorid retarded the action of the enzym so as to require more than double the time to produce the given amount of oxygen.

Sodium carbonate (anhydr.) attacks β-catalase but very slowly even in a 3 per cent solution. Even after fifteen hours at low temperature the enzym is only partially killed. To a solution of which 20 cc. produced 17.8 cc. of oxygen in fifteen minutes, 5 per cent of its bulk of sodium carbonate (anhydr.) was added in the form of a concentrated solution. After standing fifteen hours at 0° C. it was neutralized with acetic acid and, after the removal of the carbon dioxid by a current of air, 5 cc. hydrogen peroxid were added. There were still produced 7.2 cc. oxygen in fifteen minutes. More injury is caused by heating the solution for one minute with 2 per cent of sodium carbonate to 40° C, only 5 cc. oxygen being produced afterwards in fifteen minutes. Scarcely any injury is observed after fifteen hours' contact at 15° C. with a 1 per cent solution of sodium carbonate, and even after standing at the ordinary temperature for three days there is still considerable active enzym present in the solution.

No injury whatever is caused in twenty-four hours by a 2 per cent solution of sodium fluorid or by a 5 per cent solution of dipotassium oxalate. Neither a 5 per cent solution of potassium sulpho-cyanide nor of thio-urea has any apparent injurious influence upon the β-catalase. The presence of these compounds seriously interferes, however, with the reaction of the enzym upon hydrogen peroxid, since potassium sulphocyanide is rapidly oxidized by hydrogen peroxid to monopotassium sulphate and prussic acid,[1] while thio-urea is transformed into sulphuric acid and (partly) carbamide.[2] These mixtures rapidly

[1] $KCNS + 3H_2O_2 = KHSO_4 + HCN + 2H_2O$.
[2] $CSN_2H_4 + 4H_2O_2 = SO_4H_2 + CON_2H_4 + 3H_2O$.

assume a very acid reaction. In the latter case a considerable rise of temperature is noticed.[1]

BEHAVIOR TOWARD MERCURIC CHLORID.

Two grams of cured tobacco containing α- and β-catalase were left two days in 2 cc. of a 0.1 per cent solution of mercuric chlorid. While the filtrate showed no reaction whatever on hydrogen peroxid, the insoluble part still showed a slight reaction. Suspended in 30 cc. water, it yielded on addition of 10 cc. hydrogen peroxid:

Oxygen in thirty minutes.

	cc.
After treatment with $HgCl_2$	6
Control	158

This reveals a highly injurious influence of mercuric chlorid on both kinds of catalase. The β-catalase of one gram of the sample yielded 16.5 cc. oxygen in thirty minutes. Hence, even on the assumption that all β-catalase had been merely precipitated by the mercuric chlorid and thus withdrawn from the solution, and further assuming that it was in the first place the α-catalase that had been killed by that salt, an injury due to β-catalase would still be evident.

Catalase is probably also injured by various salts of other heavy metals, but the results obtained upon addition of hydrogen peroxid to such mixtures would be misleading, since many salts of these metals themselves bring on decomposition of hydrogen peroxid.

BEHAVIOR TOWARD ACIDS AND BASES.

It is a noticeable fact that while highly dilute acids retard the action of α- and β-catalase, dilute alkaline solutions promote it. In one case it was shown that on addition of 1 per cent sodium carbonate to a mixture of 10 cc. cold-prepared tobacco extract with 10 cc. hydrogen peroxid, 165 cc. of oxygen were developed in five minutes, while it took fourteen minutes to develop the same amount of oxygen when sodium carbonate was absent.

When the amount of acids in the solutions reaches more than 0.5 per cent, the α- and β-catalase are very soon killed. The following observations may be mentioned: Two grams of finely powdered sweated tobacco were shaken with 40 cc. of a 2 per cent solution of oxalic acid and the mixture neutralized after thirty minutes, with caustic soda. The catalytic power was entirely destroyed. No trace of oxy-

[1] Jacobson's inferences as to the injurious influence of potassium sulpho-cyanide upon the "catalytic property of diastase, emulsin, etc.," must be corrected accordingly, since it is the acid reaction of mono-potassium sulphate formed by the hydrogen peroxid which interferes with the result, and not the sulpho-cyanide as such. Furthermore, it is not diastase and emulsin, but a separate enzym, that causes the "catalysis of hydrogen peroxid" as here set forth.

gen was developed either by the extract or by the residue. In a second test, solutions of 0.2 per cent oxalic and sulphurous acids were permitted to act for eighteen hours under the same conditions mentioned above. The β-catalase was almost entirely destroyed and the α-catalase was also considerably injured.

The number of cubic centimeters of oxygen developed by α-catalase in thirty minutes was as follows:

	cc.
Control	158
With 0.2 per cent oxalic acid	33
With 0.2 per cent sulphurous acid	28

A solution of 0.1 per cent acetic acid at the ordinary temperature did not injure the catalytic power in one hour, but injured it gradually at a temperature of 55° C.

When sweated tobacco was suspended in fifty times its weight of a 2 per cent sulphuric acid solution the catalytic power was destroyed in fifteen minutes.[1] A solution of β-catalase lost its activity in fifteen minutes after the addition of 1 per cent sulphuric acid. α-catalase behaves the same, as was shown on suspending tobacco in fifty times its weight of 1 per cent sulphuric acid.

Saturated baryta water kills β-catalase in two days, but only slowly injures α-catalase, as shown[2] in an experiment with 2 grams cured tobacco suspended in twenty-five times its weight of baryta water. After neutralization with acetic acid and addition of 5 cc. hydrogen peroxid, the amount of oxygen developed in 15 minutes was as follows:

	cc.
α-catalase	33
β-catalase	0
Control to β-catalase	89

Sodium hydrate in a dilution of 1 per cent kills α- and β-catalase almost instantly, while a dilution of 0.1 per cent has no injurious influence, at least not within a short time.

BEHAVIOR TOWARD ALCOHOL.

Absolute alcohol injures neither α- nor β-catalase at the ordinary temperature in twenty hours. One gram of sweated tobacco was mixed with 10 cc. absolute alcohol.[3] The alcohol was filtered off

[1] It may here be mentioned that in all such cases the liquid was neutralized with dilute caustic soda before the hydrogen peroxid was added. An excess of the dilute acid had to be applied, since the tobacco often contains 10 per cent and over of salts of organic acids (malic, citric, oxalic acids).

[2] Of course a small part of the dissolved baryta was rendered inactive by combination with the acids bound to the ammonia and nicotine contained in the tobacco, but this fact, considering the large amount of baryta water, does not essentially alter the above inference.

[3] While absolute alcohol does not dissolve a trace of the enzym, alcohol of a strength of 50 per cent dissolves a considerable amount.

after twenty hours and the tobacco powder, after being freed as much as possible from the adhering alcohol by pressure between filter paper, was extracted with 20 cc. water for three hours at the ordinary temperature. The filtrate, as well as the undried residue, yielded almost exactly as much oxygen in fifteen minutes as was obtained in the control case, showing that alcohol does not act injuriously on the enzym, at least not within a short time.

A concentrated cold-prepared extract of sweated tobacco was mixed with one-tenth of its volume of alcohol and left in a filled, well-stoppered flask for five days in a dark box at a temperature of from 15° to 18° C. There was no indication of bacterial growth. Twenty cc. of this liquid gave in ten minutes 37 cc. oxygen, while in the control case 50 cc. were originally obtained. The action of catalase on hydrogen peroxid is not influenced by the presence of small quantities of alcohol, but larger quantities—above 30 per cent—exert a retarding influence, especially noticeable when the amount of catalase present is but small.

The action of boiling absolute alcohol is of some interest. One gram of sweated tobacco was kept for half a minute in boiling absolute alcohol and then, after the removal of most of the alcohol by pressure between filter paper, extracted with cold water. Upon addition of 10 cc. hydrogen peroxid to the filtrate, as well as to the residue suspended in 20 cc. water, the following results were obtained:

Cubic centimeters of oxygen developed in twenty minutes.

	By α-catalase.	By β-catalase.
Treated	55	3
Control	70	20

The remarkable fact that boiling absolute alcohol does not instantly kill the enzym is probably due in part to the fact that all enzyms resist a higher temperature in the absence of water than when dissolved in water, and also to the fact that alcohol boils at a comparatively low temperature.

BEHAVIOR TOWARD CHLOROFORM.

A concentrated aqueous extract of sweated tobacco was left with some chloroform for five days in a dark box. Then, after the evaporation of the chloroform at the ordinary temperature, 20 cc. of the liquid were mixed with 6 cc. of hydrogen peroxid. In ten minutes the volume of oxygen obtained was:

	cc.
Treated	48
Control[1]	54

[1] The control test was made with an equal amount of the same extract before the experiment was begun.

After standing twelve days another portion of the same extract had lost considerably more of its catalytic power, only 14.5 cc. of oxygen being developed in the same time.

In another trial sweated tobacco was first exhausted with water to remove all β-catalase and then left in chloroform water at the ordinary temperature for four weeks. It still showed a moderate reaction upon hydrogen peroxid, showing that some α-catalase was still present. No β-catalase had here been formed from the insoluble catalase.

The action of catalase upon hydrogen peroxid is not influenced in a marked degree by the presence of a little chloroform or ether.[1]

BEHAVIOR TOWARD PHENOL.

To an extract of sweated tobacco rich in β-catalase was added 0.2 per cent phenol, but after twenty-four hours' contact not the least decrease of catalytic power was observed. In another case the extract was completely saturated with phenol, and thus contained 6.6 per cent of it, but here every trace of catalytic power was annihilated after twenty hours.

The presence of 1 per cent phenol retards the action of the enzym. One-tenth gram of crude catalase was dissolved in 30 cc. water, and 10 cc. of this solution yielded, on addition of 5 cc. hydrogen peroxid, 25 cc. oxygen in fourteen and one-half minutes when 1 per cent phenol was present, while in the absence of the latter it took only eleven and one-half minutes to develop the same volume.

BEHAVIOR TOWARD VARIOUS OTHER REAGENTS.

The inquiry as to the nature of the activity of enzyms led the writer years ago to the assumption that in their molecules there are certain unstable atomic groupings (labil atomic groups). Such groups are on the one hand able to transform heat energy into chemical energy, and on the other hand are readily changed by atomic migration under the influence of an increased temperature or powerful compounds, such as acids, etc. The writer has expressed the view that the instability as well as the activity of enzyms might be due to the simultaneous presence of amido and aldehyde groups. In regard to the labil amido groups, their presence was rendered probable by the destructive action which formaldehyde exerts upon the power of the enzyms. As to the presence of aldehyde, or also of ketone groups, no convincing reactions could be obtained, but it remains still to be seen whether there are not aldehyde groups present in a less reactive polymerized form. The following tests with catalase were made, to characterize the labil groups:

[1] According to Jacoby, chloroform acts as a stimulant on the activity of certain oxidizing enzyms (Virchow's Archiv. vol. 157), and according to Vogel (1897) very small doses of various ethers stimulate the cellular respiration.

ACTION OF FORMALDEHYDE.

Fifty cc. of a weak alkaline solution of α-catalase were mixed with 10 cc. commercial formalin[1] previously neutralized. Of this mixture 20 cc. were immediately mixed with 5 cc. hydrogen peroxid, the amount of oxygen developed in ten minutes being as follows:

	cc.
After action of formaldehyde	3
Control	52

A diluted solution of β-catalase was mixed with 10 per cent of neutralized formalin, causing the formation of some precipitate. After twenty minutes 20 cc. were mixed with 5 cc. hydrogen peroxid and still 1 cc. oxygen was obtained, but after standing one hour no trace was produced. In the control case 44 cc. were developed in ten minutes.

ACTION OF NITROUS ACID.

Neither α-nor β-catalase is injured by a 5 per cent solution of sodium nitrite at the ordinary temperature within one day, provided the solution is neutral or weakly alkaline, but as soon as the mixture is acidulated the enzym is injured by the nitrous acid liberated.

A special test showed that 0.4 per cent free nitrous acid injured the enzym considerably in one day. One-tenth gram of crude catalase was dissolved in 100 cc. water. Chloroform was then added and the solution shaken and divided into three equal parts. One part received no addition. The second part received an addition of 0.2 gram of acetic acid, and the third part 0.2 gram of acetic acid and 0.22 gram of sodium nitrite. After standing one day these solutions were made slightly alkaline before 5 cc. of hydrogen peroxid were added. The volumes of oxygen developed in 1½ minutes were:

	cc.
Control	40
After treatment with acetic acid	8.4
After treatment with nitrous acid	2.5

The loss of activity under the influence of formaldehyde or of nitrous acid renders the supposition very probable that labil amido groups are concerned in the activity of the enzym, since labil amido groups are very readily acted upon, even in neutral and highly dilute solutions, by those compounds.[2] Here we have before us a specific action, while the loss of activity under the influence of hydrochloric, sulphuric, or

[1] The preparation contained about 40 per cent pure formaldehyde and had only a slightly acid reaction. It may be mentioned that this aldehyde in dilute and neutral solution is acted upon but slowly by hydrogen peroxid; hence this influence can be neglected here.

[2] The detrimental action of mercuric chlorid above mentioned might most easily be explained by the reaction of this salt upon labil amido groups in which hydrogen can easily be replaced by mercury. By such a chemical change of an active atomic group the activity of the enzym itself is inhibited or destroyed.

oxalic acids indicates merely the loss of lability by atomic migration without giving a decided answer as to the nature of the labil atomic groups themselves.

The next question to be considered is: By what other groups in the molecule is the lability of those amido groups induced? The following tests do not give a satisfactory answer to this question, but seem to indicate that neither ordinary aldehyde nor ketone groups are the cause. The supposition as to the presence of polymerized aldehyde groups, however, still remains to be considered, especially the detrimental action of free hydroxylamine, which might be explained by a dismemberment of a polymeric aldehyde group and subsequent reaction with the hydroxylamine.

ACTION OF PRUSSIC ACID.

One gram of tobacco containing α- and β-catalase was mixed with 20 cc. of dilute (2 per cent) prussic acid and the liquid was filtered after two hours. Ten cc. hydrogen peroxid were added to the filtrate, but no trace of oxygen was obtained. In the control case, however, 20.2 cc. of oxygen were developed in fifteen minutes. The residue in the filter was also tested after washing, but here the full original power—60 cc. of oxygen in fifteen minutes—was observed. In a second experiment the same dilute prussic acid was allowed to act on the tobacco for twenty hours, and the filtrate was then tested for the catalytic power after the prussic acid had evaporated at the ordinary temperature, but no trace of oxygen was now developed after addition of hydrogen peroxid. The α-catalase in the residue had in this case also preserved the full catalytic power. It would seem, therefore, that while β-catalase is easily killed by prussic acid α-catalase shows considerable resistance, at least as long as it is present in the undissolved state. In weak alkaline solution the result differs, inasmuch as considerable injury is caused to α-catalase by prussic acid.

A regeneration of the activity after evaporation of the prussic acid is only observed with β-catalase when the amount of prussic acid permitted to act upon it has been very small.

BEHAVIOR OF α-CATALASE TOWARD HYDROGEN SULPHID.

Two grams of finely pulverized sweated tobacco were suspended in 500 cc. of water and hydrogen sulphid passed through until complete saturation was obtained. The liquid was allowed to stand for twenty-four hours. The tobacco was then collected on a filter and after being washed well was suspended in 30 cc. of water, and 10 cc. of hydrogen peroxid added. In thirty minutes the volume of oxygen developed was 170 cc., while in the control case, after extracting the tobacco (2 grams) well with cold water, it was 609 cc. This shows

that although hydrogen sulphid injures the α-catalase at the ordinary temperature, this injury proceeds but slowly.

Schœnbein reports[1] that plant juices instantly lose their catalytic power upon coming in contact with hydrogen sulphid, but the above test shows that this power is not so easily destroyed, after all.

A second test yielded essentially the same result. In both cases a considerable amount of sulphur was set free, leading to the inference that catalase probably promotes the oxidation of H_2S by the oxygen of the air.

BEHAVIOR TOWARD HYDROXYLAMINE.

One gram of hydrochlorid of hydroxylamine[2] dissolved in a little water was neutralized with sodium carbonate, and the solution diluted to 20 cc. After the addition of 1 gram of sweated tobacco the mixture was allowed to stand for eighteen hours. The filtrate was now found to have completely lost every trace of catalytic power. This was not the case, however, with the insoluble portion, which, after washing and suspending in 20 cc. of water with the addition of 10 cc. hydrogen peroxid, developed 29 cc. oxygen in fifteen minutes. In the same length of time 62 cc. were obtained in the control case.

BEHAVIOR TOWARD PHENYLHYDRAZINE.

A concentrated cold-prepared extract of sweated tobacco[3] was salted out with ammonium sulphate, the precipitate pressed between filter paper, dissolved in a little water, and some phenylhydrazine acetate added. After twenty-four hours a small amount of precipitate was formed, which yielded some oxygen gas with hydrogen peroxid, while the filtrate gave only traces. It therefore seems that β-catalase forms a still somewhat active compound with phenylhydrazine. This, however, requires further study.

In testing α-catalase, a weak alkaline solution was mixed with some phenylhydrazine acetate. After twenty-four hours a precipitate was formed, but the amount was insignificant. The clear filtrate, upon the addition of 5 cc. hydrogen peroxid, in ten minutes yielded 11 cc. oxygen, while in the control case 52 cc. of oxygen were obtained. One would naturally suppose that if ketone or ordinary aldehyde groups were present the enzymic activity would be totally destroyed in a short time.[4]

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[1] Journ. prakt. Chem., 1863, p. 340.

[2] This salt has a strong acid reaction, hence experiments made directly with it are not conclusive. The acid reaction has to be removed, since it alone would suffice to injure or kill the enzym.

[3] Prepared with the addition of 10 per cent alcohol to prevent bacterial development.

[4] Emulsin also preserves its powers after it has been in contact with phenylhydrazine acetate for twenty-four hours. It can be precipitated with alcohol and still exhibit its action on amygdalin.

BEHAVIOR TOWARD ALKALINE SILVER SOLUTION.

A very subtile reagent for aldehyde groups is prepared by adding a few drops of dilute caustic soda to an ammoniacal silver solution which contains only a small excess of ammonia. Such a solution, containing 2 per cent silver oxid, was added to 20 cc. of the α-catalase solution above mentioned. After standing twenty-four hours a very small amount of dark sediment was formed, but the solution still had its original degree of catalytic power. A considerable separation of metallic silver and a destruction of the catalytic power would naturally have been expected were ordinary aldehyde groups concerned in the activity.

In a second test freshly precipitated silver oxid was suspended in 40 cc. of a solution of crude β-catalase (obtained by salting out tobacco extract with sodium sulphate) and the mixture tested after standing at the ordinary temperature for two days. To this and the control solution were added several drops of ether. In testing both solutions at the same time, it was found that while 20 cc. of the control solution, after the addition of 10 cc. hydrogen peroxid, developed 53 cc. of oxygen in two minutes, the filtrate from the silver oxid took three minutes to do the same; hence the injury caused by silver oxid was only moderate.

GENERAL OCCURRENCE OF α-AND β-CATALASE.

Numerous tests have established beyond a doubt that catalase is of general occurrence in the vegetable kingdom. No living plant or vegetable organ tested was found free from it, some of the plants containing more of the soluble and others more of the insoluble form. Leaves from various plant families showed principally the insoluble catalase, in some cases only slight traces of the soluble kind being noticeable, while in various seeds the soluble kind was found present in larger proportions.

Healthy green tobacco leaves, taken from the greenhouse on December 22, were dried at 40° C. After being ground to a fine powder 2 grams were extracted with 40 grams of water for three hours at the ordinary temperature. The filtrate gave reactions for oxidase and peroxidase, but showed no catalytic power on hydrogen peroxid, and therefore contained no β-catalase.[1] The well-washed residue, suspended in 20 cc. water, developed, after the addition of 12 cc. hydro-

[1] Traces of β-catalase may be observed, however, when the green tobacco leaves have not been dried, but are ground and expressed in the fresh state, the clear filtrate being directly tested. It must be borne in mind, however, that the frequent presence of nitrates in the leaves may decrease the action on hydrogen peroxid to a considerable extent, which might lead sometimes to the inference of the presence of only very small quantities of β-catalase.

gen peroxid. 107.2 cc. oxygen in fifteen minutes, and was relatively rich in α-catalase. The stalk of the tobacco plant is about as rich in this enzym as the leaves, but the fine roots contain less. Only one comparative test was made on this point, however. In the flowers (air dry) of the tobacco plant hardly any β-catalase is found, but considerable α-catalase.

Besides Phanerogams from different families, ferns, mosses, liverworts, and higher and lower algæ, as well as fungi, were also tested with positive results. It may be mentioned that vegetable cells die quickly in a 0.5 per cent solution of hydrogen peroxid, while their catalase is much more slowly injured.

Suitable data which would allow the investigator to calculate the absolute weight of catalase from a certain volume of oxygen developed in a given time and at neutral reaction are thus far not known; hence the volumes of oxygen given off by different objects can have only a relative value, serving for purposes of comparison. This is also the reason why these volumes were not reduced to normal pressure and 0° C.[1] The following tests with leaves, fruits, and seeds were carried on at the request of the writer by Mr. D. W. May. Eight grams of the leaves in the fresh state were crushed to a pulp, with the aid of a little sand and water, before the hydrogen peroxid was added, while two grams of the seeds in the air-dried state were finely pulverized, shaken up with 50 cc. of water, and the mixture left in the ice box overnight for extraction. The filtrates were gradually mixed with 5, 10, or even more cubic centimeters of hydrogen peroxid, as the degree of activity in special cases demanded, while the washed residue was suspended in from 50 to 60 cc. water before the hydrogen peroxid was added.

Cubic centimeters of oxygen developed by different leaves.

	5 minutes.	10 minutes.	15 minutes.	20 minutes.	25 minutes.
Magnolia (Magnolia sp.)	47	92	129	163	.....
Holly (Ilex sp.)	50	95	129	.....	.....
Clover[2] (Trifolium repens)	98	129	176	218	.....
Cotton (Gossypium herbaceum)	49	102	157	209	.....
Rose[2] (Rosa sp.)	52	106	156	202	252
Spruce (Picea sp.)	56	107	148	.....	.....

[1] It may be mentioned, however, that the temperature of the laboratory ranged generally from 16° to 22° C. and the barometric pressure from 746 to 771 mm.

[2] Clear filtrates of the diluted juices of the leaves of rose and clover were also prepared and tested but β-catalase was absent.

Cubic centimeters of oxygen developed by various seeds.

		Minutes.			
		5.	10.	15.	20.
GRAMINEÆ:					
Sweet corn (Zea saccharata)	Filtrate	99.0	150.0	.....	191.0
	Residue	38.0	52.0	59.0	.....
Barley (Hordeum sativum)	Filtrate	9.0	14.0	17.0	.....
	Residue	20.0	32.0	42.0	.....
Rye (Secale cereale)	Filtrate	8.0	11.4	14.0	.....
	Residue	12.5	17.0	20.0	.....
Wheat (Triticum sativum)	Filtrate	4.4	7.0	9.6	.....
	Residue	6.5	9.2	11.0	.....
Wheat (Calif.) (Triticum sativum)	Filtrate	9.0	14.0	18.0	.....
	Residue	13.1	20.1	24.0	.....
Millet (Chaetochloa italica)	Filtrate	24.0	39.0	62.0	79.0
	Residue	18.0	25.0	31.0	36.0
CRUCIFERÆ:					
Black mustard (Brassica nigra)	Filtrate	3.0	3.0	.....	.....
	Residue	9.5	12.7	14.0	.....
White mustard (Brassica alba)	Filtrate	3.2	3.5	.....	.....
	Residue	4.8	8.0	11.0	.....
Turnip (Brassica rapa)	Filtrate	1.0	1.7	.....	.....
	Residue	98.0	121.0	125.0	.....
Radish (Raphanus sativus)	Filtrate	2.0	2.4	2.6	.....
	Residue	62.0	79.0	87.0	.....
Rape (Brassica napus)	Filtrate	1.0	1.0	.....	.....
	Residue	66.0	90.0	103.0	.....
LEGUMINOSÆ:					
Peas (Pisum sativum)	Filtrate	16.0	19.0	21.0	.....
	Residue	3.6	4.0	4.2	.....
Beans (Phaseolus vulgaris)	Filtrate	62.0	82.0	95.0	.....
	Residue	20.0	28.0	35.0	.....
Lentils (Lens esculenta)	Filtrate	8.2	10.2	11.2	.....
	Residue	18.0	24.0	26.0	.....
Lupins (Lupinus luteus)	Filtrate	44.0	60.0	70.0	.....
	Residue	61.0	79.0	89.0	.....
Clover (Trifolium repens)	Filtrate	107.0	170.0	214.0	.....
	Residue	100.0	162.0	221.0	.....
Alfalfa (Medicago sativa)	Filtrate	48.0	58.0	63.0	.....
	Residue	67.0	108.0	130.0	.....
VARIOUS FATTY SEEDS:					
Cotton (Gossypium herbaceum)	Filtrate	112.0	112.5	.....	.....
	Residue	75.0	101.0	103.0	.....
Flaxseed (Linum usitatissimum)	Filtrate	59.0	88.0	96.0	.....
	Residue	66.0	90.0	95.0	.....
Sunflower (Helianthus annuus)	Filtrate	84.0	104.0	121.0	.....
	Residue	109.0	159.0	172.0	.....
Poppy (Papaver sp.)	Filtrate[1]	72.0	132.0	194.0	.....
	Residue	92.0	150.0	203.0	.....
MISCELLANEOUS SEEDS:					
Tobacco (Nicotiana tabacum)	Filtrate	50.0	70.0	81.0	.....
	Residue	32.0	45.0	54.0	.....
Beet (Beta vulgaris)	Filtrate	25.0	30.0	32.0	.....
	Residue	24.0	31.0	33.0	.....
Squash (Cucurbita sp.)	Filtrate	79.0	91.0	96.0	.....
	Residue	33.0	44.0	49.0	.....
Pumpkin (Cucurbita pepo)	Filtrate	96.0	142.0	167.0	.....
	Residue	64.0	88.0	96.0	.....
Peach (Prunus persica)	Filtrate	50.0	108.0	132.0	.....
	Residue	178.0	214.0	226.0	.....
Almonds (Prunus amygdalus)	Filtrate	22.0	36.0	48.0	.....
	Residue	5.5	7.5	9.5	.....
Chestnut (Castanea americana)	Filtrate	63.0	99.0	116.0	117.0
	Residue	68.0	89.0	96.0	.....
English walnut (Juglans regia)	Filtrate	50.0	85.0	89.0	100.0
	Residue	85.0	92.0	95.0	.....
Pecan (Hicoria pecan)	Filtrate	29.0	47.0	62.0	.....
	Residue	88.0	103.0	103.0	.....
Filbert (Corylus sp.)	Filtrate	48.0	73.0	85.0	92.0
	Residue	44.0	66.0	80.0	88.0
Butternut (Juglans cinerea)	Filtrate	15.0	29.0	38.0	.....
	Residue	7.0	11.0	14.0	.....
Peanut (Arachis hypogea)	Filtrate	117.0	123.0	124.0	.....
	Residue	72.0	97.0	110.0	.....
Peanut hull	Filtrate	3.0	5.0	8.0	.....
	Residue	6.0	10.0	12.0	.....

[1] This filtrate was not perfectly clear.

Cubic centimeters of oxygen developed by various fruits, etc

		Minutes.			
		5.	10.	15.	20.
Orange (Citrus aurantium sinensis)	Filtrate	1.5	1.8		
	Residue	2.5	2.5		
Orange II	Filtrate	2.0	1.0		
	Residue	3.0	3.2		
Orange peel	Filtrate	1.8	2.0		
	Residue	4.0	7.0	9.0	
Lemon[1] (Citrus medica limon)	Filtrate	1.0	1.5		
	Residue	.6	.8		
Lemon peel	Filtrate	1.0	1.2		
	Residue	3.2	4.5		
Banana (Musa sapientum)	Filtrate	3.0	3.0		
	Residue	6.5	10.0	13.0	14.0
Banana peel	Filtrate	5.0	7.5	10.5	
	Residue	40.0	72.0	87.0	96.0
Apple, sour (Pyrus malus)	Filtrate	4.0	4.2		
	Residue	3.0	3.0		
Apple, subacid	Filtrate	1.0	1.6		
	Residue	2.2	3.2	3.6	
Apple peel	Filtrate	3.0	3.0		
	Residue	3.0	5.0		
Apple peel, subacid	Filtrate	2.8	3.8	4.3	
	Residue	16.0	26.0	34.0	
Apple seed[2]	Filtrate	119.0	180.0	227.0	
	Residue	110.0	163.0	223.0	
Pear (Pyrus communis)	Filtrate	5.5	7.2	9.4	
	Residue	20.0	30.0	35.0	
Pear peel	Filtrate	22.0	33.0	42.0	45.0
	Residue	62.0	91.0	101.0	104.0
Date (Phœnix dactylifera)	Filtrate	4.0	7.0	8.0	
	Residue	10.5	17.5	23.0	
Strawberries, sour (Fragaria chiloensis)	Filtrate	.5	1.0		
	Residue	.5	1.0		
Strawberries, neutralized	Filtrate	6.0	7.5		
	Residue	2.0	3.5		
Onion, bulb (Allium cepa)	Filtrate	3.0	5.0	6.5	
	Residue	21.0	37.0	62.0	
Onion leaves	Filtrate	76.0	97.0	110.0	
	Residue	91.0	140.0	189.0	
Potato flesh (Solanum tuberosum)	Filtrate	56.0	82.0	92.0	98.0
	Residue	52.0	71.0	80.0	85.0
Potato peel	Filtrate	69.0	82.0	93.0	97.0
	Residue	75.0	94.0	101.0	102.0

[1] The lemon juice neutralized by carbonate of soda yielded 5 cc. oxygen in five minutes.
[2] The filtrate from apple seed developed 552 cc. oxygen in forty-five minutes, the residue 379 cc. in thirty minutes.

We learn from these determinations that the flesh of the fruits when of an acid character is poor in catalase and that at the same time the seeds may be very rich in it. Compare, for instance, the above data for apple seed and apple flesh. It is true that a better result is obtained after neutralization of the acid, but even in this case the catalytic power appears small. Compare, also, the data for strawberry and lemon. Evidently the accumulation of acid kills the enzym. We notice further a relatively large amount in the fatty seeds, but in starchy seeds only the sweet corn and chestnut are noticeably rich in catalase. Relatively a very large content of β-catalase is observed with seeds of poppy and clover. Turnip, radish, and rape seed, while rich in α-catalase, are exceedingly poor in β-catalase. During germination the total catalase is very much increased, as observed with barley shoots about 5 cm. long. Twenty grains yielded in twenty minutes 30 cc. of oxygen, while twenty shoots from the same lot yielded fully 130 cc.

In fungi the amount of catalase is relatively very large. Spores of *Penicillium glaucum* were sown in one liter of a sterilized aqueous solution of the following:

	Per cent.
Peptone	0.1
Glucose	.5
Malic acid	.2
Dipotassium phosphate	.1
Magnesium sulphate	.01

After four weeks a large mass of mycelium with spores was developed, which was filtered off. In the clear yellowish filtrate, which now showed a neutral reaction, a considerable reaction for β-catalase was obtained, but none for either oxidase or peroxidase.[1] Of this filtrate three portions of 50 cc. each were taken. The first was mixed directly with 10 cc. of hydrogen peroxid; the second portion only after heating for two minutes at 75° C. and quickly cooling; the third portion after heating to 87° for one minute and allowing to cool slowly. The volume of oxygen developed in fifteen minutes from the first portion was 78 cc., from the second portion only 14.8 cc., while from the third portion no oxygen at all was developed. The washed mycelium, together with the spores, was now left for one hour in 80 per cent alcohol in order to kill the mycelium, which was then pressed gently between filter paper, dried at 40° C., and finely pulverized. Of this product 0.5 gram was again washed well with water, in order to remove all β-catalase, and then suspended in 20 cc. water. Hydrogen peroxid in portions of 10 cc. was now gradually added. The sudden start and sudden stop of the development of oxygen gas was very striking and evidently indicated the presence of a great deal or of a very energetic kind of catalase. When the process had slowed down considerably further addition was stopped. In the short time of forty-one minutes fully 800 cc. of oxygen were developed[2] from 90 cc. of hydrogen peroxid added. It was probably the α-catalase of the mycelium only and not that of the spores of the fungus that caused this considerable development, since the membranes of the latter are impregnated by a fatty substance that admits aqueous solutions to the interior with considerable difficulty.[3]

Another fungus tested was *Pleurotus sapidus*, which proved very rich in β-catalase but less rich in α-catalase. Conidia of a Uredo also showed a very powerful action.

A test for catalase in bakers' yeast yielded the following result: A portion was divided into three equal parts, one part being dried at

[1] A slight blue coloration that set in after half an hour's standing in the test for oxidase (not peroxidase) can hardly be considered as a decisive test.

[2] Barometric pressure 771 mm.; temperature 22° C.

[3] That the accumulation of β-catalase in a liquid is not inimical to bacterial growth was proven with the mold culture filtrates.

110° C. to determine the dry matter, which was found to be 1.851 grams; another portion, suspended in 25 cc. of water, after the addition of 10 cc. of hydrogen peroxid developed 82 cc. of oxygen in thirty minutes; the third part was left with 100 cc. of a 10 per cent glucose solution for twenty-four hours and, after filtering the fermenting liquid, the filtrate and residue were tested separately. The filtrate gave no reaction with hydrogen peroxid; the residue treated as above gave 108 cc. of oxygen in thirty minutes. This sample of yeast contained no oxidase and but a trace of peroxidase.

Another test was made with fresh beer yeast, the yeast being free from any trace of oxidase and containing only a faint trace of peroxidase. A portion of the yeast was suspended in 100 cc. of water and with frequent shakings the mixture was divided into two equal parts, one part serving for the determination of the dry matter, which was found to be 0.540 gram. The other part was left with 3 cc. of chloroform for twenty-four hours in order that the cells might be killed and consequently give up their enzyms to the water more easily, thus making it possible to determine whether there was any soluble catalase present. This was found to be the case. The clear filtrate gave, upon the gradual addition of 15 cc. of hydrogen peroxid, 117 cc. of oxygen in thirty minutes. The killed yeast itself, however, after washing gave 452 cc. of oxygen in fifteen minutes. This hardly leaves any doubt but that the amount of α-catalase here is larger than the amount of β-catalase. It is noticeable also that the fresh beer yeast is richer in total catalase than bakers' yeast.

Various bacteria also produce considerable catalase,[1] *Bacillus pyocyaneus*, for example; but the amount depends to some extent upon the conditions of nutrition. In a nutrient fluid containing 0.5 per cent peptone and 1 per cent glycerol or cane sugar as organic materials there was much less catalase produced than in a solution containing 1 per cent glycerol, 0.2 per cent tyrosine, and 0.2 per cent sodium acetate, but no peptone.

In the animal kingdom catalase is also of general occurrence. The aqueous extracts of spleen, pancreas, liver, kidney, brain, muscles, and also the blood serum show the power of catalyzing hydrogen peroxid, but certain secretions, as, for example, the urine, lack it. Infusoria, insects, worms, and mollusks were examined also with positive results.

The luminous parts of a lightning beetle do not contain a markedly larger proportion of catalase than other parts of this insect. Different parts of about equal size were crushed with about 10 cc.

[1] Other oxidizing enzyms—oxidase and peroxidase—do not appear to be produced by many bacteria. Cultures of *B. subtilis* and *B. pyocyaneus* did not show them, but according to Roux (Comptes Rend., vol. 128, p. 212, 1899), *B. coli* can produce one of these.

water, and after transferring into an Erlenmeyer flask, 5 cc. hydrogen peroxid were added (rendered previously slightly alkaline). The time necessary to produce 20 cc. and 40 cc. oxygen was then noted. The results were:

Oxygen.	With head.	With upper abdomen.	With luminous parts.
	Minutes.	*Minutes.*	*Minutes.*
20 cc	1½	1¾	1¼
40 cc	5¾	7¼	5½

A small beetle of about the size of the head of the lightning beetle produced 40 cc. oxygen in six and one-fourth minutes, while a medium-sized *Carabus*, 0.618 gram in weight, after being crushed with 20 cc. water, produced, upon the gradual addition of 20 cc. hydrogen peroxid, 168 cc. oxygen in four minutes, corresponding to the decomposition of nearly 0.5 gram pure anhydrous hydrogen peroxid.

The extracts[1] of animal organs yield, on saturation with ammonium sulphate, precipitates which, after drying at the ordinary temperature, are only partly soluble again in water. However, not only the soluble portion, but also that which has become insoluble, shows catalytic power. Two-tenths of a gram of the insoluble part of a crude catalase preparation from calf's spleen, when suspended in 20 cc. water, yielded, upon addition of 5 cc. hydrogen peroxid, 29.2 cc. oxygen in twenty minutes. Hence this product contains relatively little of the active principle. An aqueous extract of beef muscles has a high degree of catalytic power, and even after it is heated for one moment to 62° C. and the coagulated albumen is removed the clear filtrate has still a powerful action on hydrogen peroxid, while the coagulum itself gives merely a faint trace of action, showing that it absorbed only traces of catalase. Finely chopped beef extracted for fifteen minutes with twice its weight of water at 40° C. yielded a reddish filtrate of weak acid reaction, of which 20 cc. decomposed 5 cc. of a 3 per cent hydrogen peroxid preparation almost completely in one minute. When rendered slightly alkaline the same amount of filtrate produced, upon addition of 20 cc. hydrogen peroxid, fully 163 cc. oxygen gas in but two minutes.

The presence of α-catalase as well as β-catalase in the pancreatic gland becomes probable from the fact that the pulp shows considerable catalytic power even after repeated extraction with chloroform water. A 1 per cent solution of sodium carbonate at the ordinary temperature, however, extracts the insoluble portion of catalase but slowly, and besides transforms a part of it into β-catalase by its prolonged influence. The hen's egg is an object poor in catalase. Ten cc. of the white of an egg, upon mixture with 5 cc. hydrogen peroxid, yielded

[1] These extracts were prepared by the digestion of the finely chopped organs with water at 40° to 50° C. for a short time and filtering.

only 21 cc. of oxygen in fifteen minutes, and the entire yolk yielded only 1 cc.

Milk sometimes contains only traces of catalase. In one case 20 cc. of fresh cow's milk gave, upon the addition of 10 cc. hydrogen peroxid, only 2.4 cc. oxygen in fifteen minutes.[1] In another instance 20 cc. of fresh milk yielded, after the addition of 15 cc. hydrogen peroxid, 9.2 cc. oxygen in the same length of time.[2]

Some observations made by Spitzer[3] with regard to the amounts of oxygen produced by various organs may be added here:

Oxygen developed in—	Dog.				Cattle.	Frog.
	Blood (1 gram).	Liver (1 gram).	Brain (2 grms.).	Muscles (2 grms.).	Pancreas (1 gram).	Ovaries (2 grms.).
	cc.	cc.	cc.	cc.	cc.	cc.
4 minutes	85	65	30.8	22	71	24
13 minutes	.....	76	56	54	82	48

IS CATALASE AN OXIDIZING ENZYM?

The simple fact that catalase energetically decomposes hydrogen peroxid would not in itself justify the inference that catalase is also an oxidizing enzym. Such a deduction has, however, some probability, since we find this catalytic property together with the power of inducing oxidations in one other substance, viz, platinum black. But platinum black furthermore shows the chief characteristic of the ordinary oxidase—that of causing, even in the absence of hydrogen peroxid, a blue reaction with guaiac, which reaction is not obtained with catalase. With platinum black even reducing actions are known,[4] the writer having years ago shown that nitrates are reduced to ammonia by glucose in aqueous solution upon the addition of some very active platinum black.[5] In this process the oxygen of the nitrates is thrown upon the sugar, which is thus converted into acids, while hydrogen from the sugar migrates to the nitrogen of the nitrates, yielding ammonia. This interesting process, which demonstrates that an agency causing oxidations can under other circumstances also cause reductions, seems to have remained unknown to some recent writers, to judge from their peculiar discussions of the reducing actions in the living cells.

[1] Since catalase and other oxidases are not constant concomitants of milk, it might be of interest to compare, in this regard, milk from healthy with that from diseased animals.

[2] The so-called test of Storch to distinguish boiled from unboiled milk has nothing to do with catalase.

[3] Pflüger's Archiv, vol. 67 (1897).

[4] Berichte der Deutschen Chemischen Gesellschaft, Vol. XXIII, p. 675 (1890).

[5] The decrease of the nitrate content during the sweating process of tobacco and the formation of nitrite (and probably of ammonia) thereby may be due to an analogous action of catalase. A short contact, however, does not suffice to accomplish such a change, as a special test showed.

In how many particulars, however, the behavior of platinum black is again encountered in catalase will need to be determined by special investigations. Some authors hold the oxidases to be transferrers of oxygen, but in reality they are transferrers of chemical energy by which certain other compounds are enabled to take up the molecular oxygen directly, which they could otherwise do only at a high temperature. Spitzer ascribes to the iron organically bound in certain nucleo-proteids the property of inducing oxidations. The ordinary oxidase and peroxidase, however, as well as β-catalase, do not possess the general characteristics of nucleo-proteids, but those of albumoses.

The action of oxidizing enzyms in animals has been investigated by a number of authors. It has been found that a pulp made from various animal organs, or extracts prepared therefrom, can oxidize salicylic aldehyde, benzaldehyde, and formaldehyde to their corresponding acids. Methyl alcohol can be oxidized to formic acid, benzyl alcohol to benzoic acid, and the oxidation of acetone and uric acid has also been observed. Various color reactions[1] caused by oxidation, as the formation of indophenol, Bindschedler's green, and totuylene-blue,[2] are also, according to Spitzer, induced by the animal oxidases. Of inorganic materials the oxidation of arsenious to arsenic acid by animal juices was observed by Binz.

It appears that several oxidizing enzyms exist in animals. Pohl prepared extracts which were capable of oxidizing aldehydes but incapable of giving the indophenol reaction. Jacoby observed the destruction of uric acid by dog's liver, but not by calf's liver. We encounter further contradictory statements, especially in regard to the oxidation of glucose and arabinose by the expressed juices of liver and pancreas. Jacoby found the amount of arabinose thus oxidized to be very small.[3] W. Spitzer and others have observed the destruction of a small amount of glucose by blood drawn from animals and by various extracts of animal organs.[4] This so-called glycolysis was ascribed to an oxidizing enzym which, according to Spitzer, gives a blue reaction

[1] These color reactions are best obtained by moistening paper with the reagents and bringing it in contact with the animal pulp.

[2] The colored compounds of Lauth can not, however, be formed by oxidases (Spitzer).

[3] This author further observed that the animal oxidase which easily oxidizes salicylic aldehyde to salicylic acid, is not destroyed at 70° C., and that small quantities of chloroform, or a solution of 0.1 per cent sodium hydrate, stimulate its action, while 1 per cent sodium carbonate, or 0.3 per cent sodium hydrate, prevent it.

[4] Pflüger's Archiv, vol. 60, p. 303 (1895). As examples he mentions that 10 cc. dog's blood can destroy in one hour, at 40° C., 20 milligrams of glucose (determined by titration); 25 cc. defibrinated dog's blood oxidized (completely?) at 39° C., 0.1 gram of glucose in twenty-four hours. The serum is not active; the cellular elements contain the active principle (Lépine, Spitzer). Addition of hydrogen peroxid did not promote the process of destruction, not even upon further addition of palladium, which produced nascent oxygen from the hydrogen peroxid.

with guaiac on addition of hydrogen peroxid, and also shows at the same time catalytic action on hydrogen peroxid. This opinion, however, had already been doubted by Jacoby, who assumes not only one but several oxidizing enzyms in the animal, and also holds that the agency oxidizing the sugar is different from that which oxidizes salicylic aldehyde to salicylic acid.[1]

Quite recently F. Umber[2] has asserted that the destruction of sugar by an extract of the pancreas gland is merely due to microbes, since it is not observed when antiseptic measures are resorted to.

The inability of α- and β-catalase to produce a blue reaction with guaiac solution appears at first sight to be decisive against the assumption that these enzyms can bring on any oxidation; but this inference is not justifiable, since the action of the oxidizing enzyms is sometimes quite specific; that is, they act only on a certain group of bodies of a distinct chemical character or on compounds in which not only a certain degree of lability, but also a configuration, is found that coincides in a certain measure with that of the oxidizing enzym. Grüss has recently observed an oxidizing enzym in barley grains which has no reaction on guaiac, but yields a violet color with tetramethylparaphenylendiamine. He calls it spermase.[3]

The indophenol reaction also can not be produced by catalase. This was tried with a preparation from sweated tobacco and with one obtained by salting out the filtrate of a culture of *Penicillium* by ammonium sulphate. A trace of hydrogen peroxid in the control case sufficed to produce this blue color in the mixture of paraphenylendiamine and α-naphthol in presence of sodium carbonate, even in the absence of oxidizing enzyms.

A characteristic oxidation by catalase, however, is produced with hydroquinone, the odor of quinone being developed within a very short time. This has been tried with β-catalase salted out from the juice of potatoes, with extract of poppy seed, with a concentrated extract of sweated tobacco, and, further, with tobacco that had been well extracted with water containing chloroform. This well-washed tobacco, containing a considerable amount of α-catalase, gave, after being moistened with a dilute solution of hydroquinone and standing fifteen minutes, the characteristic quinone odor, which after

[1] Virchow's Archiv, vol. 157, p. 235 (1899).

[2] Zeitschrift für Klinische Medicin, vol. 39, p. 13 (1900).

[3] Sometimes the reaction of the medium to be tested has to be carefully observed, since certain color reactions, as, for example, the indophenol reaction, succeed only in weak alkaline solution. Sometimes certain compounds may also be present which interfere with the production of the reaction. Thus, Epstein observed that the action of the oxidase contained in fresh juice of beets is prevented by the presence of prussic acid, and is restored when this is expelled by a current of air. This is also a decisive proof of the enzymatic nature of the oxidizing phenomena in fresh beet juice. (Archiv für Hygiene, vol. 36, p. 140.)

twenty-four hours became very strong. The same moist tobacco was heated in a closed vessel to 95° C. for ten minutes and then moistened with the same solution, but no trace of the quinone odor could be perceived, even after twenty-four hours. The same negative behavior was observed with tobacco cured with artificial heat at a higher temperature and therefore containing no catalase (flue-cured bright-yellow Virginia tobacco), no trace of the odor being produced.

The sweated tobacco which was used in the tests with hydroquinone was free from oxidase and peroxidase and did not contain any other known enzym;[1] it therefore follows that α- and β-catalase belong to the class of oxidizing enzyms.

The smell of quinone produced by the action of animal catalase upon hydroquinone is less marked, perhaps on account of the presence of too large a proportion of impurities of a protein nature. But this oxidation can be observed well when to a concentrated extract of beef, made at the ordinary temperature, 1 per cent of hydroquinone is added and the mixture is left for several days in a large flask plugged with cotton. The hydroquinone produces a precipitate and also a gradual and moderate darkening of the mixture. It furthermore prevents, in that concentration, bacterial development. When after several days a little oxalic acid is added, a very marked odor of quinone is developed, becoming much stronger on heating.

While certain oxidases can oxidize very dilute eugenol to vanillin, catalase seems incapable of accomplishing this; at least no odor of vanillin was noticeable after one day. Nor is ethyl alcohol oxidized by it either to aldehyde or to acetic acid, and cyanin is not decolorized by it, although this compound is easily destroyed by other oxidizing agents, as, for example, hydrogen peroxid. Saligenin and indigo carmin are not attacked by it, at least not in neutral solution.

Other tests were made to ascertain whether carbonic acid would be produced from various organic compounds by the action of α-catalase. Sweated tobacco, rich in α-catalase, was well extracted with chloroform water at the ordinary temperature, pressed, and an amount corresponding to from 35 to 40 grams of dry matter put in a flask, after being moistened with solutions of the organic substances to be tested, and heated to from 55° to 60° C. in a current of air, which had passed through a solutionof caustic potash and then through a flask containing chloroform. For absorption of the carbonic acid a solution of barium hydrate containing 0.62 per cent of BaO was used. Each test lasted one hour. Control tests were also made, since not only the absorption of carbonic acid from the air by the tobacco before it was placed in the flask, but

[1] It is scarcely necessary to state that the common hydrolyzing enzyms—diastase, emulsin, papain, etc.—have not the power of oxidizing hydroquinone to quinone. The writer has especially assured himself on this point.

also the oxidation of certain organic materials contained in the leaves, was possible.[1]

It was found that of the 25 cc. of baryta water applied in the two control cases there were neutralized 3.1 and 3.4 cc., respectively. The other results were as follows:

Compound.	Concentration.	Baryta water neutralized.
	Per cent.	*cc.*
Sodium malate	6	3.5
Sodium tartrate	6	5.0
Sodium citrate	6	9.1
Tyrosine	1	4.0
Nicotine sulphate	1	3.5
Soap	10	3.5
Glucose	20	6.2

During the one hour the test lasted no distinct traces of ammonia were produced either from tyrosine or nicotine. These compounds might, however, show less resistance under the influence of other oxidation processes of a considerable activity as, for instance, in the fermenting of the tobacco piles. The amount of carbonic acid produced in this short time was also but small, and may represent a mere by-product of partial oxidations. Some oxidation in the above cases can only be assumed for glucose and citric acid, since the other numbers differ too little from those of the control case.

Another special test showed that an aqueous solution of 0.5 per cent glucose, in presence of 1 per cent phenol as an antiseptic, is not changed by β-catalase at the ordinary temperature within a fortnight. The solution was kept in a large closed flask in contact with much air, and was repeatedly titrated with Fehling's reagent. The result may differ, however, when the mixture is exposed to the air on a porous surface and in presence of an alkaline reaction.

THE PHYSIOLOGICAL IMPORTANCE OF CATALASE.

There does not exist a group of organisms or any organ or even a single vegetable or animal cell that does not contain some catalase, as far as the observations of the writer go. This general occurrence of catalase in the organized world can not be accidental and must have a certain significance. Since the destruction of hydrogen peroxid is the most characteristic property of catalase, can this property be of physiological value? Is there any production of hydrogen peroxid in the living cells, and, if so, is the destruction of this product of advantage to the cells? The catalase of the cells would energetically destroy any trace of this compound immediately after its formation (see also

[1] By the previous extraction with water, fatty matter, nucleo-proteids, and a portion of the coloring matter could not, of course, be removed.

p. 17.) The possibility of the production of hydrogen peroxid in the living cells while the energetic oxidation representing the respiration process is going on, can not be denied; it is, in fact, very probable. Recent investigations have shown beyond a doubt that labil hydrogen atoms in an organic compound can form hydrogen peroxid on coming into contact with free oxygen. When it happens that the main organic complex is otherwise not readily oxidized, and especially is not easily changed further by the hydrogen peroxid formed, the amount of the latter produced can be determined quantitatively. Thus it can be shown that this amount corresponds to the amount of the labil hydrogen atoms which have separated from the organic compound to combine with molecular oxygen. Such a compound is phenylhydroxylamine, studied by Eugen Bamberger.[1] It has a relatively stable atomic structure, the two unstable atoms being the two hydrogen atoms in the hydroxylamine group, which are inclined to combine directly with one molecule of oxygen when exposed to the air in aqueous solution.

$$\underbrace{C_6H_5NHOH}_{\text{Phenylhydroxylamine.}} + \underbrace{O{=}O}_{\text{Molecular oxygen.}} \text{ gives } \underbrace{C_6H_5NO}_{\text{Nitrosobenzene.}^2} + \underbrace{H{-}O{-}O{-}H}_{\text{Hydrogen peroxid.}}$$

In presence of sodium hydrate the hydrogen peroxid formed is rapidly consumed again, the nitrosobenzene being thereby oxidized to nitrobenzene. Hence the primary formation of hydrogen peroxid in this latter case escapes notice, which also happens in numerous other instances of autoxidation. Cases exist, however, where even in presence of sodium hydrate the hydrogen peroxid remains unchanged, as shown by W. Manchot.[3] Oxanthranol dissolved in alkalies forms a red solution which in contact with air is rapidly decolorized and separates white anthraquinone, while at the same time hydrogen peroxid is produced.

$$\underbrace{C_6H_4\begin{cases}CHOH\\CO-\end{cases}C_6H_4}_{\text{Oxanthranol.}} + O{=}O \text{ gives } \underbrace{C_6H_4\begin{cases}CO\\CO\end{cases}C_6H_4}_{\text{Anthraquinone.}} + H{-}O{-}O{-}H$$

It was found by that author that for one molecule of anthraquinone one molecule of hydrogen peroxid was produced, which confirms the correctness of this equation. Quite in analogy is the transformation of dihydrophenanthrenquinone to phenanthrenquinone, that of hydrochrysoquinone and hydroretenquinone to their respective ketones, and that of hydrazotriazol to azotriazol.

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[1] Berichte der Deutschen Chemischen Gesellschaft, vol. 33, p. 113 (1900).

[2] The nitrosobenzene thus primarily formed is acted upon by still unchanged molecules of phenylhydroxylamine and forms azoxybenzene. Three grams of phenylhydroxylamine in 40 cc. water yielded, after treatment with a current of air for seventy hours, 2.5 grams azoxybenzene and 0.398 gram hydrogen peroxid, which corresponds to 92.5 per cent of the theoretical yield.

[3] Ueber freiwillige Oxydation, Leipzig, Veit & Co., 1900.

Hence the view of Traube that *nascent* hydrogen atoms can reduce molecular oxygen to hydrogen peroxid can also be extended to *labil* hydrogen atoms.

The transformation of molecular oxygen into hydrogen peroxid is considered as an activifying of oxygen. In analogy with the formation of hydrogen peroxid the peroxids of organic complexes also may be formed in certain processes of autoxidation, as Engler[1] has recently shown for pinen, amylen, hexylen, styrol, cyclopentadiën, and diallylether. Benzaldehyde yields by autoxidation, according to Baeyer,[2] primarily benzoylated hydrogen peroxid, $C_6H_5—CO—O—O—H$, which easily transfers 1 atom of oxygen upon another molecule of benzaldehyde, or upon some other easily oxidizable material, and thereby becomes benzoic acid. This would, however, not yet justify an attempt to extend the analogy also upon the oxidations caused by the living protoplasm, since it would involve a continuous oxidation of the protoplasm itself and lead to an early death of the cells before reconstruction of the injured parts would become possible. Nevertheless the proteids composing the living matter betray an aldehyde nature by the toxicological behavior of the living matter.[3]

The oxidations in the living protoplasm have often been ascribed to an activified oxygen, and doubtless many chemists would feel inclined to assume the formation of hydrogen peroxid in order to account for oxidations of which common oxygen seems incapable. But it must be considered that the great lability of the proteids of the living protoplasm—in other words, the accumulation of kinetic chemical energy in them—is here a more important factor than the natural tendency of common oxygen or hydrogen peroxid to cause oxidation. It is further a fact that hydrogen peroxid can not be utilized by the living protoplasm as an oxidizer, since the active atomic groups in the proteids of the living matter would be oxidized by it instead of the thermogens stored up in the cells for combustion, and injury or death would result; indeed the highly poisonous character of hydrogen peroxid is well known.[4] Therefore, since the oxidation in the respiration process would take a detrimental turn if hydrogen peroxid accumulated as a by-product, the rôle of the catalase may possibly be explained. It would destroy every trace of this poisonous substance as quickly as it were formed and would thus afford an important protection. The

[1] Berichte der Deutschen Chemischen Gesellschaft, vol. 33, p. 1103.

[2] Ibid., p. 1582.

[3] These points are fully discussed in chapter 12 of the treatise of the writer, Die chemische Energie der lebenden Zellen, Munich, 1899, E. Wolff, publisher.

[4] In a dilution of 1: 200 hydrogen peroxid kills cholera bacilli in three minutes; of 1: 15,000 it impedes development of typhoid bacilli; of 1: 10,000 it kills infusoria in from fifteen to thirty-six minutes; and in one of 1: 1,000 algæ are killed in a short time. Intravenous injections kill mammals by stopping respiration.

oxygen set free by this destructive operation could at once be utilized again for the continuance of the respiration process.[1]

It may be asked what function the catalase performs in fermenting yeast and anaërobic microbes, since in these there is no normal respiration process and hence no occasion for the formation of hydrogen peroxid by autoxidation.[2] Catalase must then have still another function, and the writer is inclined to assume for it the faculty also of loosening chemical affinities in certain compounds to such a degree that the protoplasm itself can more easily split them, or, when oxygen has access, can more easily oxidize them. In other words, catalase might represent an aid for fermentative as well as as for respirative phenomena. In the case of the yeast it may be of importance for the action of Buchner's zymase. The amount of catalase in yeast is relatively large (see p. 35), and the expressed juice of yeast is also rich in it. Catalase may also be capable of accomplishing certain reducing processes, since platinum black can also induce reductions as well as oxidations (see above, p. 37). An enzym with a reducing action was recently observed by Abelous and Gérard in the aqueous extract of the horse kidney. It is capable of reducing nitrates to nitrites, and nitrobenzene to aniline.[3]

Since catalase is of universal occurrence in the organized world, and is capable of destroying every trace of hydrogen peroxid as soon as it is formed, it is quite impossible that the latter product could ever be found in living cells. The assertions, therefore, of Clermont, Wurster, Bach, and E. Baumann to the contrary have lost every trace of justification. Direct tests by Bokorny, Pfeffer, Cho, and others, have proved the incorrectness of this assumption.[4] It is evident, further, that Reinke's hypothesis of the respiration process,[5] which assumes the formation of hydrogen peroxid as a necessary intermediate step to enable the combustions to take place in the protoplasm, must be abandoned. The protoplasm would hardly prepare a special enzym

[1] Some authors go so far as to ascribe the entire respiration process to the activity of certain enzyms. This view is certainly incorrect; otherwise the oxidations characteristic of the respiration process should continue qualitatively and quantitatively, after heating cells to their death point (40°–50°C).

[2] Among the obligate anaërobic microbes the bacillus of blackleg was tested and also found to contain catalase.

[3] Académie des Sciences, meeting of December 11, 1899.

[4] It may also be mentioned that Schoenbein's assertion of the occurrence of hydrogen peroxid in the violet fluorspar of Wölsendorf has been refuted, since the writer demonstrated long ago that Schoenbein's results are not due to the presence of that supposed "antozon," or to hydrogen peroxid, but to traces of free fluorin, probably formed by the gradual dissociation of small quantities of a superfluorid of cerium. The sentence on p. 113, Vol. XIII, of the "Handwörterbuch" relating to Schoenbein's observations should therefore be corrected.

[5] Botanische Zeitung, 1883, Nos. 5 and 6.

that would destroy with great vigor and promptness a product necessary for its respiration.

The protoplasm represents a most complicated machine, built up of easily changeable proteids. This easy change to comparatively stable forms, in the process of dying, implies a loss of the labil atomic groups by atomic migration in the molecules, and a loss of kinetic chemical energy.[1] The atoms of the labil groups are in continuous motion, representing a charge with kinetic chemical energy. This energy is transferred by the protoplasm to the thermogens, principally sugar and fat (lecithin), which at the ordinary temperature are not at all disposed to oxidize themselves directly on contact with air. By contact with the living protoplasm, however, they become capable of thus oxidizing themselves, since the protoplasm imparts to their atoms such a state of motion that their affinities are loosened and they can take up oxygen from the air without the oxygen being previously activified. This splitting of the oxygen molecules and the combining of the oxygen atoms with the atoms in sugar and fat proceeds at one and the same moment; but at the same time certain labilized activified hydrogen atoms of the thermogens can combine with still entire molecules of oxygen, thus forming hydrogen peroxid as a by-product, as previously mentioned.

The heat produced by the combustion of the thermogens is again turned in a certain measure by the living protoplasm into chemical energy, since the heat energy instigates the atoms of the labil groups to much more intense oscillations than is the case with the more stable atoms. The easy transformation of heat energy into chemical energy is known. The former represents motions of molecules and atoms, the latter an atomic motion which consists of oscillations of a greater amplitude than the motions induced by heat energy. This can be inferred from the fact that, other things being equal, chemical energy is capable of loosening affinities in molecules much more readily than heat energy can at a moderate temperature.

When the thermogens are consumed in the cell without a fresh supply being available, the labil proteids of the living protoplasm themselves take up oxygen. Thus by their partial oxidation a disturbance is produced which may, at a certain stage, lead to the total collapse of the protoplasm with a loss of all the labil groups. This is the death of the cells by starvation.

When, however, thermogens are present but the oxygen is withheld, the activified molecules of sugar undergo other changes, brought on by the far-reaching loosening of affinities, and lactic acid, alcohol, or fat result, accompanied by a simultaneous production of

[1] This energy is perceptible as heat in the dying of the muscular tissues of animals—the so-called postmortem rise of temperature.

carbonic acid from destroyed sugar molecules. This process, which in most cases is of but short duration and is terminated by the early death of the cells by suffocation, is known under the name of "intramolecular respiration." The recent important observations of Eugen Bamberger furnish an interesting chemical analogy to ordinary and intramolecular respiration. He found that β-phenylhydroxylamine not only oxidizes itself readily on contact with air and induces oxidation of other compounds, as indigo-carmine, but can also, in the presence of a little alkali and the absence of air, change itself to aniline and nitrosobenzene, which represents on the one side a reduction and on the other an oxidation, in full analogy to the changes in intramolecular respiration.

The development of oxygen by the action of catalase upon hydrogen peroxid recalls another instance of development of oxygen; it is the assimilation of carbon. Here the oxygen developed is generally assumed to be derived directly from carbonic acid, as expressed by the following equation:

$$\underbrace{O=C\begin{matrix}\diagup OH\\ \diagdown OH\end{matrix}}_{\text{Carbonic acid.}} \text{ becomes } \underbrace{O=C\begin{matrix}\diagup H\\ \diagdown H\end{matrix}}_{\text{Formic aldehyde.}} + \underbrace{O_2}_{\text{Molecular oxygen.}}$$

An ingenious hypothesis of Erlenmeyer[1] tries, however, to explain this separation of oxygen by a previous formation of hydrogen peroxid and assumes formic acid to be the first organic product of the assimilation process.

$$O=C\begin{matrix}\diagup OH\\ \diagdown OH\end{matrix} + \begin{matrix}H\\ |\\ OH\end{matrix} \text{ becomes } \underbrace{O=C\begin{matrix}\diagup OH\\ \diagdown H\end{matrix}}_{\text{Formic acid.}} + \underbrace{HO-OH}_{\text{Hydrogen peroxid.}}$$

$$\begin{matrix}HO-OH\\ HO-OH\end{matrix} = 2H_2O + O_2$$

Since the formation of formic acid is not very probable, we might substitute the following equation which would express an eventually direct formation of formic aldehyde, which by condensation may yield sugar.

$$O=C\begin{matrix}\diagup OH\\ \diagdown OH\end{matrix} + \begin{matrix}HOH\\ HOH\end{matrix} \text{ becomes } O=C\begin{matrix}\diagup H\\ \diagdown H\end{matrix} + \begin{matrix}H_2O_2\\ H_2O_2\end{matrix}$$

$$2H_2O_2 = 2H_2O + O_2.$$

Pfeffer[2] gives the following declaration against the above hypothesis: "Erlenmeyer supposed that formic acid and hydrogen peroxid are formed during the assimilation of carbon dioxid—a theory which the absence of hydrogen peroxid from assimilating plants conclusively negatives." The undeniable fact that all reagents fail to show the

[1] Berichte der Deutschen Chemischen Gesellschaft, 1877, p. 634.

[2] Physiology of Plants, Vol. I, p. 356 (1900.)

presence of hydrogen peroxid in the cells can not, however, be accepted by a critical mind as a proof that it is not formed at all, since it would have only an ephemeral existence. By its rapid decomposition it would give rise to the oxygen developed by the chlorophyll bodies—a view which would find some support if it could be shown that the chlorophyll bodies are very rich in catalase.

While Pfeffer's objection can not be considered a serious one, some better-founded chemical objections might be raised. It is a fact that chemistry does not show a single instance where two hydroxyl groups can unite to form hydrogen peroxid. Such a process would be improbable, although not impossible.[1]

[1] Brühl (Ber. d. Deutschen Chem. Ges., vol. 33, p. 1710) maintains that hydrogen peroxid can not be a simple "bihydroxyl" HO—OH, but that the formula HO ≡ OH would correspond better with its behavior, since it is free of electric absorption and shows a very high dielectric constant.